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Article (Published Version)

Access the most recent version at http://jeb.biologists.org/lookup/doi/10.1242/jeb.167304

The interaction of path integration and terrestrial visual cues in navigating desert ants: What can we learn from path characteristics?

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Key words

Navigation, Multimodal interactions, Path integration, Visual guidance, Ants

Summary statement

The combination of large-scale path recordings with high-speed recordings at key locations allows us to analyse path characteristics of navigating ants under the influence of path integration and visual guidance.

Abstract

Ant foragers make use of multiple navigational cues to navigate through the world and the combination of innate navigational strategies and the learning of environmental information is the secret of their navigational success. We present here detailed information about the paths of *Cataglyphis fortis* desert ants navigating by an innate strategy, namely path integration. Firstly, we observe that the ants' walking speed decreases significantly along their homing paths, such that they slow down just before reaching the goal, and maintain a slower speed during subsequent search paths. Interestingly, this drop in walking speed is independent of absolute home-vector length and depends on the proportion of the home vector that was completed. Secondly, we find that ants are influenced more strongly by novel or altered visual cues the further along their homing path they are. These results suggest that path integration modulates speed along the homing path in a way that might help ants search for, utilise or learn environmental information at important locations. Ants walk more slowly and sinuously when encountering novel or altered visual cues and occasionally stop and scan the world, this might indicate the re-learning of visual information.

Introduction

A vital task in the life of a scavenging desert ant is to safely return to a rather inconspicuous nest entrance after extensive foraging journeys (Buehlmann *et al.* 2014; Huber & Knaden 2015). The clever combination of innate navigational strategies and the learning of information from the environment is the secret of their navigational success (Wehner 2003; Collett *et al.* 2013; Knaden & Graham 2016). *Cataglyphis* desert ants do not lay pheromone trails but individually navigate using path integration and information from the environment such as visual (Wehner *et al.* 1996; Huber & Knaden 2015) or olfactory cues (Buehlmann *et al.* 2012; Buehlmann *et al.* 2014, 2015). Path integration is an innate behaviour allowing exploration of unfamiliar terrain while keeping track of direction (Wehner & Müller 2006) and distance travelled (Wittlinger *et al.* 2006) to maintain an estimate of the direct path back to the origin of the journey (Müller & Wehner 1988; Collett & Collett 2000; Wehner & Srinivasan 2003; Ronacher 2008). It is essential when unfamiliar with the environment, but as a strategy, it is prone to cumulative errors (Sommer & Wehner 2004; Merkle *et al.* 2006) and may have to be followed by systematic search if the nest is not located (Wehner & Srinivasan 1981; Schultheiss & Cheng 2011). For accurate route guidance and homing, ants complement path integration with visual information learnt from panoramic scenes (Collett *et al.* 2007; Graham & Cheng 2009; Wystrach *et al.* 2011a; Lent *et al.* 2013; Buehlmann *et al.* 2016). Visual information can be used to pinpoint the nest (Wehner & Räber 1979; Wehner *et al.* 1996; Narendra *et al.* 2007b) and guide habitual routes (Collett *et al.* 1992; Kohler & Wehner 2005; Collett 2010; Wystrach *et al.* 2011b). Indeed, the learning of visual cues for route guidance allows ants to robustly travel between the nest and a feeding site along idiosyncratic routes (Collett *et al.* 1992; Wehner *et al.* 1996; Graham *et al.* 2003; Kohler & Wehner 2005; Wystrach *et al.* 2011b; Mangan & Webb 2012).

Path integration itself involves little or no learning (Narendra *et al.* 2007a; Merkle & Wehner 2009) but plays an important role in the learning of visual information. Path integration can be used to guide specific exploration behaviours which facilitate the learning of visual information (Judd & Collett 1998; Nicholson *et al.* 1999; Wehner *et al.* 2004; Müller & Wehner 2010; Stieb *et al.* 2012; Fleischmann *et al.* 2016; Fleischmann *et al.* 2017). Similarly, ants using path integration in unfamiliar terrain will take consistent and direct paths, potentially simplifying the learning of visual information along a route (Collett *et al.* 2003). So even though visual cues can later be retrieved and utilised independently of the state of path integration (Collett *et al.* 1992; Collett *et al.* 2001; Kohler & Wehner 2005; Mangan & Webb 2012), path integration may provide a crucial scaffold for visual learning (Graham *et al.* 2010; Müller & Wehner 2010).

In experienced ants, visual guidance and path integration are redundant navigational strategies, and behavioural experiments have shown that ants can make simultaneous use of these multiple sources of navigational information (Narendra 2007b; Reid *et al.* 2011; Collett 2012; Legge *et al.* 2014), perhaps even weighting the cues optimally based on their reliability (Wystrach *et al.* 2015). One way of looking at interactions between path integration and the use of terrestrial visual cues is to investigate ants' paths when the direction indicated by the path integration system is at odds with the information from visual cues. We can see situations where ants strongly weight either visual (see e.g. (Narendra *et al.* 2013a)) or path-integration information (e.g. (Wehner *et al.* 1996)). However, ants often chose a compromise direction when path integration and familiar visual scenes are in a subtle conflict (Collett *et al.* 2007; Collett 2012; Wehner *et al.* 2016). Likewise, we can learn from experiments where ants with a path integration home vector are displaced to a location outside of their habitual route, such that learnt and current visual scenes do not match. In such situations, ants' paths are less accurate and more sinuous and ants follow path integration for only a proportion of the home vector length before starting systematic search (Fukushi & Wehner 2004; Beugnon *et al.* 2005; Narendra 2007b; Buehlmann *et al.* 2011; Wystrach *et al.* 2011b; Cheng *et al.* 2012; Cheung *et al.*

2012; Schultheiss *et al.* 2016). These studies are further evidence of interesting interactions between path integration and visual guidance, although the detailed nature of such interactions are unclear.

Our aim here is to look at path characteristics of navigating ants under the influence of path integration and visual guidance. Across three experiments with a combination of traditional recordings of full paths with high-speed recordings at key locations, we analyse ants' path characteristics for different path-integration states and in response to novel and familiar visual cues. As previously reported, walking speed (see e.g. (Zeil *et al.* 1996; Narendra *et al.* 2013b; Degen *et al.* 2015; Schultheiss *et al.* 2015)), pausing behaviour (see e.g. (Narendra *et al.* 2013b; Wystrach *et al.* 2014; Zeil *et al.* 2014)) and path straightness (see e.g. (Buehlmann *et al.* 2011; Wystrach *et al.* 2011b)) can provide insights into the interaction of path integration and visual guidance. Our key new findings are that (i) the ants' walking speed decreases significantly along homing paths and stays lower during subsequent search paths and (ii) ants are influenced more strongly by novel or learnt visual cues the further along their homing path they are.

Material and methods

Species and study site

All experiments were performed with the North African desert ant, *Cataglyphis fortis*, in a salt pan (34.954897 N, 10.410396 E) near the village of Menzel Chaker, Tunisia. Experiment 1 was performed with ant foragers from only one nest while in Experiments 2 and 3 multiple nests were used.

Experiment 1: Walking speeds during homing paths and nest searches

We trained foragers to travel back and forth between their nest and a feeder, that was placed either 5 m, 10 m or 20 m away (Fig. 1A), using biscuit crumbs provided *ad libitum*. For test recordings, individuals were taken from the feeder and paths were recorded on a distant test field. A grid of lines (mesh width: 1 m) was painted on the ground and the ants' paths were recorded on squared paper with additional positions noted every 5 secs. Here, and everywhere else, each ant was recorded only once. Each path was cut at the position where the ant switched from a straight homing

path to systematic nest search. This switch point was found by breaking the path into 0.5 m chunks and finding the first chunk that differed by at least 90° from the feeder-nest direction (see also (Merkle *et al.* 2006; Buehlmann *et al.* 2011; Schultheiss & Cheng 2012)). Thus, we classify path segments before the turning point as part of the homing path and segments after this point as part of the nest search and these were analysed separately. To control for speed differences due to unknown variables, such as ants' body sizes, the size of the biscuit crumb or the temperature, we normalised walking speed during both homing and nest search to an individual ant's mean homing and search speed respectively. For homing paths, these normalised speed values were associated to relative path positions. To do this, for each path the distance immediately before search is set to 1 to account for individual ants having slightly different path lengths. Paths were then divided into 10 bins and bins filled with the corresponding speed values. If an ant provided multiple values per bin because of a low walking speed or sinuous path, the median was taken. To analyse the effect of path sinuosity on walking speed during search, the index of straightness (i.e. beeline / total path length) was calculated for each path segment. Path segments during search were considered as straight when their index of straightness was as high as the mean index of straightness of that ant's homing path.

Experiment 2: Observing ants' responses to visual novelty

As in Experiment 1, paths from ants taken from a feeder at either 5 m, 10 m or 20 m from the nest were recorded on the test field. A change in the visual environment was created by adding an unfamiliar recording setup that consisted of a 0.6 m x 0.6 m wooden board (arena hereafter) placed on the ground with a camera tripod next to it and two barriers (each 1.5 m long, approximately 4 cm high) that were attached at 45° to the corners of the arena to guide the ants onto it (Fig. 1B). For the 5 m training condition, we had an extra test condition where we increased the visual mismatch by additionally adding two black boards (each 1 m long, 0.5 m high) behind the arena (see Fig. S2D). Homing ants were released on the test field at specific locations such that they had completed either 20%, 50% or 80% of their homing paths when they reached the centre of the arena. For instance, an ant taken from the feeder that was 5 m away from the nest was released either 1 m (20% of path completed when crossing the arena), 2.5 m (50% of path completed) or 4 m (80% of path completed)

away from the centre of the arena. Similarly, ants from the feeders that were 10 m and 20 m away from the nest were released at the corresponding locations. Paths preceding the arena were recorded on paper and once the ants were on the arena their paths were recorded with a high-speed camera (see below for details). As a control, paths from ants that were familiar with the setup at either 20%, 50% or 80% were recorded on the training site. Because the natural spread of the paths was wider than the dimension of the recording setup, we only considered ants in our analyses that arrived within the area enclosed by the two barriers. For the ants that entered this area, we counted those that kept walking in the homing direction and crossed the arena versus those that turned around and made a detour. If they crossed the arena we analysed if any U-turns occurred prior to entering the arena. For this purpose, paths were broken into 0.2 m segments and we determined if any chunks differed by at least 90° from the feeder-nest direction. From the high-speed recordings, we extracted the ants' average walking speed (i.e. full path length / total time), index of straightness (i.e. beeline / full path length) and the frequency of pauses per 1 m of path. Pauses are defined as periods along the path where walking speed drops below 0.1 ms^{-1}.

Experiment 3: Observing interactions between path integration and visual guidance

In this experiment, the ants' food-ward and homeward training routes slightly differed. The 10 m route to the feeder was over open ground but the first section of the return to the nest was through an aluminium channel that was either 1 m or 6 m long (height, 7 cm; width, 7 cm). Therefore ants had either completed 10% or 60% of their homing path when reaching the channel exit. At the channel exit, they crossed the 0.6 m x 0.6 m arena and passed a cylinder (height, 0.41 m; width, 0.45 m) 0.8 m to the left of the direct feeder-nest path (Fig. 1C). Ants were tested in three situations: control (Contr.), cylinder shifted 2 m leftwards (Shift.) and cylinder missing (Miss.). All tests were implemented on the familiar training ground. Paths were recorded on paper once the ants had left the channel and with the high-speed camera (mounted over the last part of the channel), to record ants crossing the arena. Walking speed, index of straightness and occurrence of pauses were extracted from the high-speed recordings as described above.

High-speed recordings and data processing

Using a Panasonic DMC-FZ200 camera we could record portions (60 cm by 60 cm) of ants' paths at 200 fps. The trajectories were extracted from videos using Ctrax (Version 0.5.3; http://ctrax.sourceforge.net/) and the associated Matlab toolbox (BehavioralMicroarray) (Branson *et al.* 2009) and corrected for tilted perspectives. When using the high-speed camera the field of view was a large wooden board (arena) with calibration marks to enable the calibration of the camera for aspect and position. To exclude the effects of the arena edges on ants' behaviour, the outer 2 cm was excluded from analysis. Large scale paths were recorded on paper (with time stamps) and digitized using GraphClick (Version 3.0; http://www.arizona-software.ch/graphclick/). All data was processed and analysed in Matlab.

Results

Walking speed decreases along ants' homing paths

In order to investigate the relationship between walking speed and path-integration state we recorded homing paths of ants returning from a feeder that was either 5 m, 10 m or 20 m away from the nest (see Fig. 1A). As described many times for *C. fortis*, when released on the test ground ants run off their path-integration vector before switching into a systematic nest search with loops centred on the fictive nest position (see e.g. (Wehner & Srinivasan 1981)). When looking at the speed characteristics, we firstly see a general trend that walking speed starts high and then significantly decreases preceding the search (Fig. 2A). The ants' speed in the final path segment immediately preceding the commencement of search (medians: 5 m ants, n = 15 ants, 0.19 ms^{-1}; 10 m ants, n = 14 ants, 0.17 ms^{-1}; 20 m, n = 18 ants, 0.18 ms^{-1}) is significantly lower than the max speed (medians: 5 m ants, 0.34 ms^{-1}; 10 m, 0.36 ms^{-1}; 20 m, 0.32 ms^{-1}) along the homing path (Wilcoxon matched pairs test: for all three training distances, $P < 0.001$). Ants reached this max walking speed at 32% (5 m ants: total path length, median, 5.0 m), 33% (10 m ants: total path length, median, 8.9 m) and 50% of their homing path (20 m ants: total path length, median, 18.4 m) respectively. Interestingly, when

comparing the speed profiles across entire homing paths for the three groups we see a consistent trend that at around 85% of the homing path ants are walking significantly slower than during the majority of the route (Fig. 2A), i.e. speed profiles seem to be independent of the absolute home-vector length.

Walking speed is lower during nest search

At the end of their straight homing paths on the test field, ants switch to a systematic nest search, and during this nest search, ants walk consistently slower than during the straight homing paths (Fig. 2B; Wilcoxon matched pairs test: 5 m and 10 m ants, both $n = 14$ ants, $P < 0.01$; 20 m ants, $n = 18$ ants, $P < 0.001$). Interestingly, the lower speed is not simply caused by paths being more sinuous. We separated search paths into straight and curved sections and the walking speed during straight portions of the search is still significantly lower than the walking speed during the straight homing path (Wilcoxon matched pairs test: 5 m and 10 m ants, $P < 0.01$; 20 m ants, $P < 0.001$). As previously reported for systematic nest searches (Wehner & Srinivasan 1981), we do not observe a change in speed across the duration of the recorded search paths (Spearman r correlation, all $P \geq 0.05$).

Tolerance for visual novelty decreases along the homing path

Having observed that ants guided by path integration reduce their walking speed along their homing path, we next looked at the ants' tolerance for visual novelty. Ants taken from the feeder were released on the test field where we had placed a small arena, with barriers and a camera tripod. The visually novel setup was placed such that ants arrived at it after having completed 20%, 50% or 80% of their homeward path (see Fig. 1B). We assessed the ants' response to this visual novelty by asking whether they would continue to follow their path integration indicated direction and by analysing fine-grained path details. On comparing the test ants that were unfamiliar with the setup with control ants that were familiar with it, we observe significant differences in path characteristics. In the presence of visual novelty, ants less often cross the arena and more often perform U-turns prior to crossing (Fig. 3). Moreover, in the presence of novel visual cues, ants walk slower (Fig. S1A), pause more often (Fig. S1B) and their paths are less straight (Fig. S1C). Interestingly, when increasing the

visual mismatch by adding even more unfamiliar visual cues (see Fig. S2D), we see a trend that speed and index of straightness drop further and the frequency of pauses further increases (Fig. S2).

We next analysed the effect of path-integration state on the ants' response to visual novelty. We found that the longer ants had travelled before arriving at the novel setup, the less likely they were to cross the arena (Fig. 3A, top; Chi-square test for trend; 5 m and 20 m, $P < 0.001$; 10 m, $P \geq 0.05$). There is also a trend for U-turns to be more frequent with increasing distance from the release point (Fig. 3A, bottom; Chi-square test for trend; 10 m, $P < 0.05$; 5 m and 20 m, $P \geq 0.05$).

Response to learnt visual cues increases along the homing path

After demonstrating that ants' paths are more disturbed when modifications in the visual world are experienced further along their homeward path, we next looked at the interactions of path integration and visual guidance by learnt visual cues. Ants were trained with a cylinder located at either 10% or 60% of their homing path (for differences in walking speed see Fig. S3A). In tests, we moved the cylinder 2 m to the left or removed it entirely (see Fig. 1C). When the cylinder was moved ants' paths shifted left also, both in terms of initial heading direction at the channel exit (Watson-Williams tests with Bonferroni corrected P value of 0.0167; 10% of path completed: Fig. 4A, Contr. vs. Shift., $P < 0.0167$, Contr. vs. Miss., $P \geq 0.0167$, Shift. vs. Miss., $P \geq 0.0167$; 60% of path completed: Fig. 4B, Contr. vs. Shift., $P < 0.0167$, Contr. vs. Miss., $P \geq 0.0167$, Shift. vs. Miss, $P \geq 0.0167$) and in terms of ants' lateral position when level with the cylinder (Kruskal-Wallis tests with Dunn's multiple comparison tests; 10% of path completed: Fig. 4A, contr. vs. shift., $P \geq 0.05$, Contr. vs. Miss., $P < 0.01$, Shift. vs. Miss., $P \geq 0.05$; 60% of path completed: Fig. 4B, Contr. vs. Shift., $P < 0.01$, Contr. vs. Miss., $P \geq 0.05$, Shift. vs. Miss., $P \geq 0.05$). Both at the channel exit and cylinder level, ants that already have completed 60% of the homing path showed a greater shift than ants that had only completed 10% of their path (Mann-Whitney tests; both $P < 0.05$), i.e. ants respond more strongly to changes in the learnt visual cue, the closer they are to the nest.

As we might predict from Experiments 1 and 2, fine-grained details of the paths reveal that walking speed and index of straightness drop with increasing distance along the homing path, while the number of pauses increases (Fig. S3). Looking closely at paths that contain pauses, we see that walking speed and frequency of pauses are to some extent independent (see Fig. S3D) and in around half of the paths with pauses (21 out of 46 ants) we additionally observe scanning, i.e. a rotation of the ants' body axis during a period of no translation (c.f. (Narendra *et al.* 2013b; Wystrach *et al.* 2014; Zeil *et al.* 2014)). There is also a non-significant trend for changes in the visual scene to have greater impact on walking speed in ants that had completed 60% of their homing path (medians: Contr., 0.24 ms^{-1}, Shift., 0.21 ms^{-1}; Mann-Whitney test: P = 0.056) than in ants that only had completed 10% of their path (medians: Contr., 0.33 ms^{-1}, Shift., 0.30 ms^{-1}; Mann-Whitney test: P = 0.934).

Discussion

We have presented information about the paths of *Cataglyphis fortis* desert ants navigating by path integration, and from this we can highlight two primary findings. Firstly, we observe that ants' walking speed decreases significantly along their homing paths, such that they slow down just before reaching their goal (Fig. 2A), and maintain this slower speed during their subsequent search paths (Fig. 2B). Our second result is that ants respond more strongly to novel or altered visual cues the further along the homing path they are (Figs 3, 4). Lower walking speeds are associated with a higher frequency of pauses and more sinuous paths (Figs 3, S1, S2, S3). Low walking speeds, more pauses and meandering paths mean that ants have more time to respond to other sensory cues at locations closer to the nest. This might suggest that path integration modulates homing paths in a way that helps ants search for, utilise or learn other sensory information such as visual (Wehner & Räber 1979; Bregy *et al.* 2008) or olfactory (Steck *et al.* 2009; Buehlmann *et al.* 2012) cues. As well as highlighting some of the subtle details of cue integration in navigating ants, this work also highlights the value of fine-grained descriptions of behaviour in naturalistic conditions.

Does the modulation of walking speed help ants to weight guidance cues?

Effective navigation is a multimodal process taking into account information from different sources (reviewed in (Wehner 2003; Collett *et al.* 2013; Knaden & Graham 2016)) and is tuned to the ants' sensory ecology (Fukushi 2001; Fukushi & Wehner 2004; Beugnon *et al.* 2005; Narendra 2007b; Narendra 2007a; Buehlmann *et al.* 2011; Wystrach *et al.* 2011b; Cheng *et al.* 2012; Schultheiss *et al.* 2016). It is well described that ants often follow visually-defined routes when visual cues are at odds with path integration (Wehner *et al.* 1996; Kohler & Wehner 2005; Mangan & Webb 2012; Narendra *et al.* 2013a). However, we also know that navigational strategies can be used simultaneously (Narendra 2007b; Bregy *et al.* 2008; Reid *et al.* 2011; Collett 2012; Legge *et al.* 2014), and moreover ants might even be able to weight cues optimally based on their reliability (Legge *et al.* 2014; Wystrach *et al.* 2015). For instance, it has been shown that the weighting of the directional component of path integration gets stronger for longer path-integration vectors (Wystrach *et al.* 2015) which matches the mathematical prediction of smaller angular variance for path integration over larger distances (Vickerstaff & Cheung 2010). Interestingly, we find a similar result in our experiments. Ants modulate their walking speed in such a way that they produce lower speeds near the fictive goal location (Fig. 2), i.e. where there is a shorter path-integration vector. It is at these positions that ants also respond more strongly to visual cues (see Figs 3, 4). Thus, path integration mediated path characteristics might assist ants in adequately responding to other sensory cues at locations of importance, by allowing those other cues to act for a longer period of time. Furthermore, by reducing speed before the fictive nest visual cues might be used before the ant overshoots the nest into the less familiar part of the world (Müller & Wehner 1988; Wystrach *et al.* 2013).

Does the modulation of walking speed allow ants to learn visual cues?

As well as during navigation by experienced foragers, navigational modalities also interact during learning. Innate navigational strategies such as path integration, pheromone trails and innate responses to ecologically relevant stimuli can all facilitate learning (Voss 1967; Collett 1998; Heusser

& Wehner 2002; Collett *et al.* 2003; Graham *et al.* 2003; Collett 2010; Graham & Wystrach 2016). Learning walks are a particularly well-studied example involving path integration (Judd & Collett 1998; Nicholson *et al.* 1999; Wehner *et al.* 2004; Graham & Collett 2006; Müller & Wehner 2010; Stieb *et al.* 2012; Fleischmann *et al.* 2016; Fleischmann *et al.* 2017). During these choreographed movements, ants have ample, well-structured opportunities to learn visual scenes (Judd & Collett 1998; Graham *et al.* 2010; Müller & Wehner 2010), and there are other motor behaviours seemingly related to learning. *Melophorus* ants produce scanning behaviours along routes triggered by unfamiliarity or uncertainty (see e.g. (Wystrach *et al.* 2014)), i.e. where new information is needed. Modulation of walking speed as observed in the current study (see Figs 2, S3), might be a similar mechanism to facilitate learning at important or novel locations. Indeed, there is ample evidence of interactions between visual learning and/or guidance and walking or flying speed. For instance, it is reported that flight speed during learning flights increases with distance away from the nest in wasps (Zeil *et al.* 1996) and flight speed increases with increasing number of orientation flights in bees (Degen *et al.* 2015). For ants, walking speed drops when visual information is harder to extract (Narendra *et al.* 2013b) and ants in tandem pair walks have lower walking speeds than solitary foragers (Schultheiss *et al.* 2015). In all these examples the lower walking speed seems to positively correlate with the amount of visual learning required, or the difficulty of the task.

We have seen that desert ants guided by path integration modulate their speed as they travel along their homing path and search for their nest. Moreover, the further along the homing path ants are the stronger they respond to novel and altered visual cues. Similarly, earlier work has shown that ants respond more strongly to visual or olfactory nest cues the further along the homing paths they are when encountering them (Michel & Wehner 1995; Bregy *et al.* 2008; Buehlmann *et al.* 2012). It seems that path integration produces reduced speeds at important locations when other cues might be important and walking speed could be an indirect mechanism for weighting cues or facilitating learning. This general trend is backed-up by changes of walking speed seen in *Cataglyphis* ants mounted on a track ball (Dahmen *et al.* 2017) and by increases in pausing and scanning in *Melophorus* and *Myrmecia* species triggered by spatial uncertainty (Narendra *et al.* 2013b; Wystrach

et al. 2014; Zeil *et al.* 2014). Encountering novel or altered visual cues may trigger re-learning of route information, hence, the observed changes in the ants' movements might facilitate the required learning. Of course, as we have suggested above, modulation of path properties might also relate to cue weighting. These possibilities are not mutually exclusive, indeed we cannot fully separate the learning and use of sensory cues. Further research is needed to gain a better understanding about cue integration and to unpick the circularity between multimodal learning and the use of multimodal cues during navigation. Finally, by providing evidence that path characteristics, like walking speed, might modulate the weighting and/or learning of environmental cues we show the importance of looking at the fine-grained sensori-motor details of navigating ants under naturalistic conditions.

Acknowledgements

We thank Roman Huber and Markus Knaden for logistical support in the field and Tom Collett for comments on a previous version of the paper.

Competing interest

No competing interests declared.

Funding

The research leading to these results has received funding from the people Programme (Marie Curie Actions) of the European Union's Seventh Framework Programme (FP7/2007-2013) under REA grant agreement no: [PIEF-GA-2013-624765] and a fellowship from the Swiss National Science Foundation (grant no. P2SKP3-148476) to C.B..

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Figures

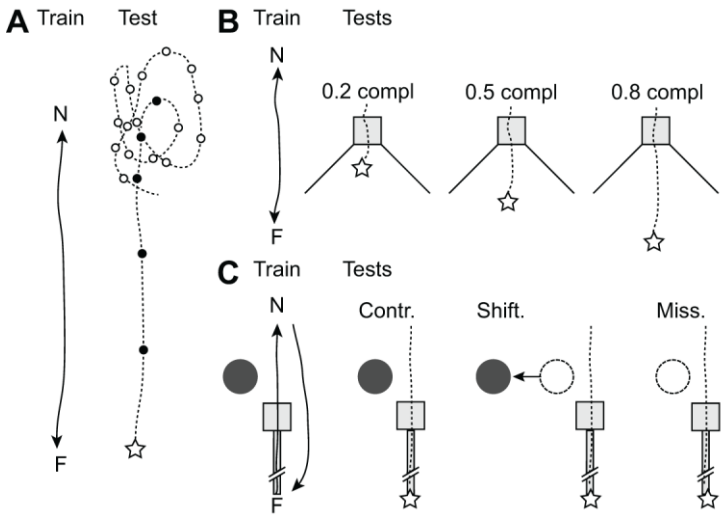

Figure1:

Experimental configuration for training and test conditions. Nest, N. Feeder, F. Training paths, black solid lines with arrows. Homing paths in tests, black dotted lines. Point of release in tests, asterisk. **A)** Exp. 1: Relationship between walking speed and path-integration state. Ants were trained to a feeder that was either 5 m, 10 m or 20 m away from the nest. Homing paths of ants taken at the feeder were recorded on a distant test field. 5 sec time intervals were marked simultaneously (circles). Homing (filled circles) and search paths (empty circles) were analysed separately. **B)** Exp. 2: Response to visual changes along homing paths. Ants taken from feeder at 5 m, 10 m or 20 m were released on the test field such they had completed either 20%, 50% or 80% of the homing paths when reaching the centre of the arena (grey square). Test paths were recorded from point of release to edge of arena. Visual change comes from the barriers that guided ants onto the arena and the tripod placed next to it. **C)** Exp. 3: Interaction between path integration and learnt visual cues along homing paths. Training distance, 10 m. During training and subsequent tests, a channel (height, 7 cm; width, 7 cm; grey rectangle) was either 1 m or 6 m long, i.e. ants had completed either 10% or 60% of their homing path when reaching the exit of the channel and entering the arena (grey square). Circle depicts cylinder. Three test conditions: control (Contr.), cylinder shifted 2 m leftwards (Shift.), Cylinder missing (Miss.). Paths were recorded from channel exit to nest.

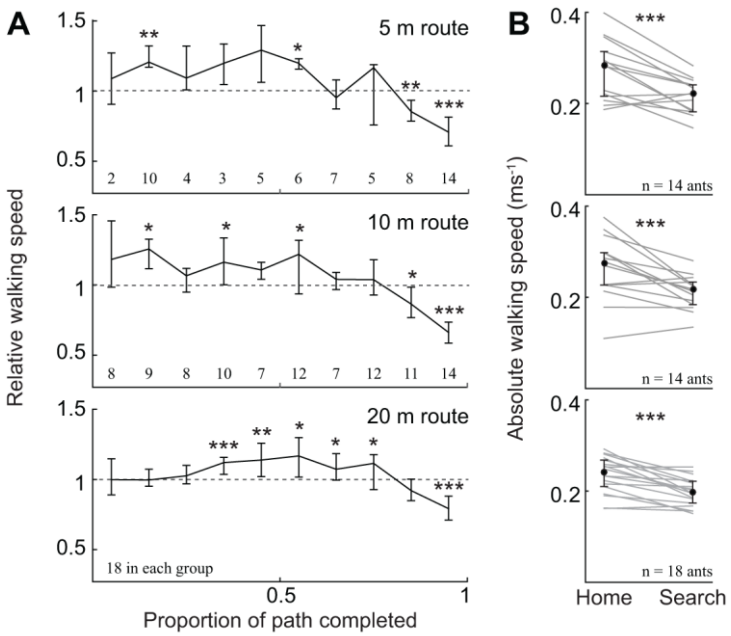

Figure 2:

Walking speed decreases along the homing path and is lower during nest search. A) Relative walking speed along homing paths for ants with either 5 m, 10 m or 20 m homeward routes (see Fig. 1A). For each ant, walking speed was normalised to that ant's mean homing speed and each speed measurement was associated to a relative path position. For each path the distance at end of the homing path is set to 1 and speed data is filled into 10 equal bins. Data plotted as medians with error bars showing 25th and 75th percentiles. Asterisks indicate where data is significantly different from 1 (Wilcoxon tests: *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$). Numbers below error bars show number of ants that contributed to each bin. **B)** Mean absolute walking speed during nest search was compared with mean homing speed. Data is plotted as medians with error bars showing 25th and 75th percentiles for homing (Home) and search paths (Search) and grey lines show changes for individual ants. Asterisks indicate significant differences using Wilcoxon matched pairs test (***, $P < 0.001$).

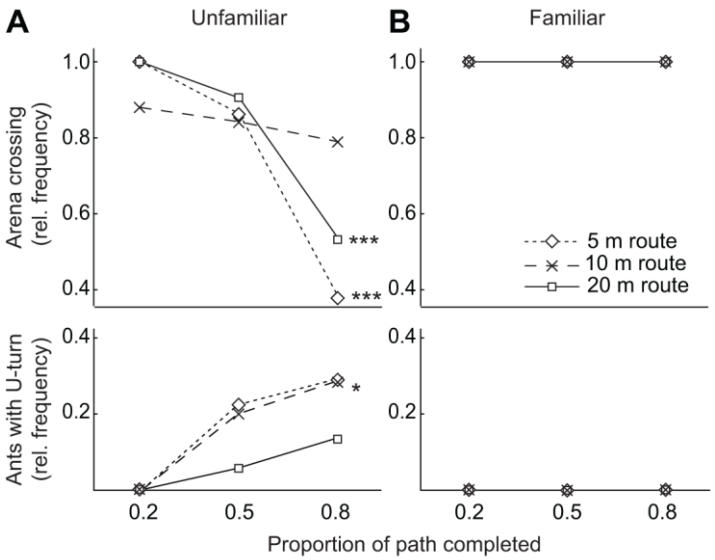

Figure 3:

Ants' tolerance for visual novelty decreases along homing paths. A) Relative frequency of ants crossing the arena (top) or performing U-turns prior to crossing (bottom) when unfamiliar with the visual cues. Ants with either a 5 m, 10 m or 20 m homeward route were released such that they encountered the visual cues when having completed either 20%, 50% or 80% of the homing path (see Fig. 1B for experimental setup). Asterisks indicate significant differences along homeward paths using Chi-square test for trend (***, $P < 0.001$; *, $P < 0.05$). 5 m: 0.2 of path completed (n = 14 ants, crossing = 14, U-turns = 0); 0.5 of path completed (n = 22 ants, crossing = 19, U-turns = 4); 0.8 of path completed (n = 29 ants, crossing = 11, U-turns = 3). 10 m: (25, 22, 0); (19, 16, 3); (19, 15, 4). 20 m: (28, 28, 0); (21, 19, 1); (15, 8, 1). **B)** As (A) but for ants that were familiar with the visual cues. 5 m: (36, 36, 0); (31, 31, 0); (30, 30, 0). 10 m: (14, 14, 0); (15, 15, 0); (13, 13, 0). 20 m: (10, 10, 0); (13, 13, 0); (11, 11, 0). Ants encountering visual novelty differed from control ants that were familiar with the visual cues in arena crossing (Fisher's exact test; 5 m: 0.2 of path completed, $P \geq 0.05$; 0.5 of path completed, $P \geq 0.05$; 0.8 of path completed, $P < 0.001$; 10 m: all $P \geq 0.05$; 20 m ants: $P \geq 0.05$; $P \geq 0.05$; $P < 0.05$) and occurrence of U-turns (Fisher's exact test; 5 m: $P \geq 0.05$; $P < 0.05$; $P < 0.05$; 10 m: all $P \geq 0.05$; 20 m ants: all $P \geq 0.05$).

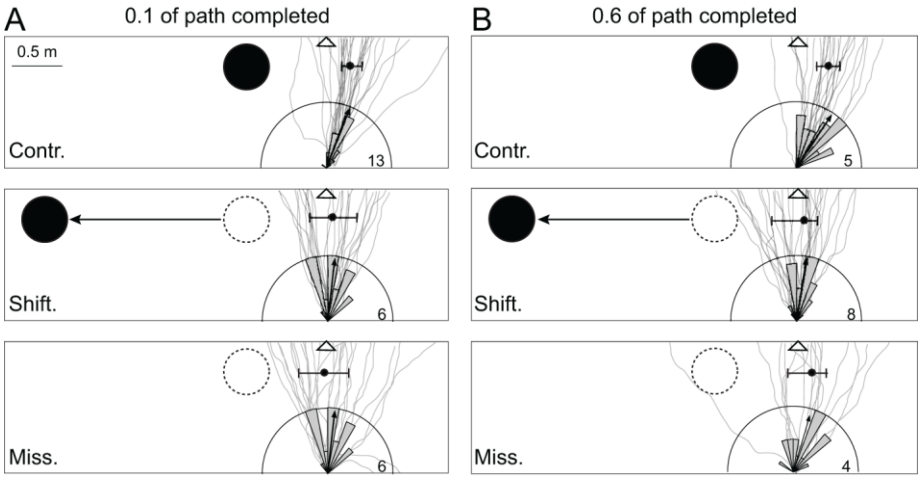

Figure 4:

Interactions between path integration and learnt visual cues. A) Homing ants had completed 10% of the homing path when reaching the channel exit (see Fig. 1C for experimental setup). **B)** Ants had completed 60% of homing path at channel exit. Initial heading direction after 20 cm from channel exit, trajectories from individual ants, and lateral position when level with the cylinder are shown for test conditions. Contr., control. Shift., cylinder shifted 2 m leftwards. Miss., Cylinder removed. Circle depicts cylinder and triangle the direction towards the nest. Initial heading directions are shown in circular histograms (bin size, 10°). The number on circle edge indicates scale for the circular histogram. Arrow within the histogram represents the mean vector. Note, circular histograms are not shown to the same scale as the trajectories and cylinder position. Lateral positions at cylinder level are plotted as medians with error bars showing 25th and 75th percentiles. For 0.1 of path completed: Contr., n = 29 ants / 30 ants (shown in histogram / shown in error bar); Shift., n = 27 ants / 26 ants; Miss., n = 27 ants / 26 ants. For 0.6 of path completed: Contr., n = 24 ants / 24 ants; Shift., n = 31 ants / 31 ants; Miss., n = 17 ants / 17 ants.

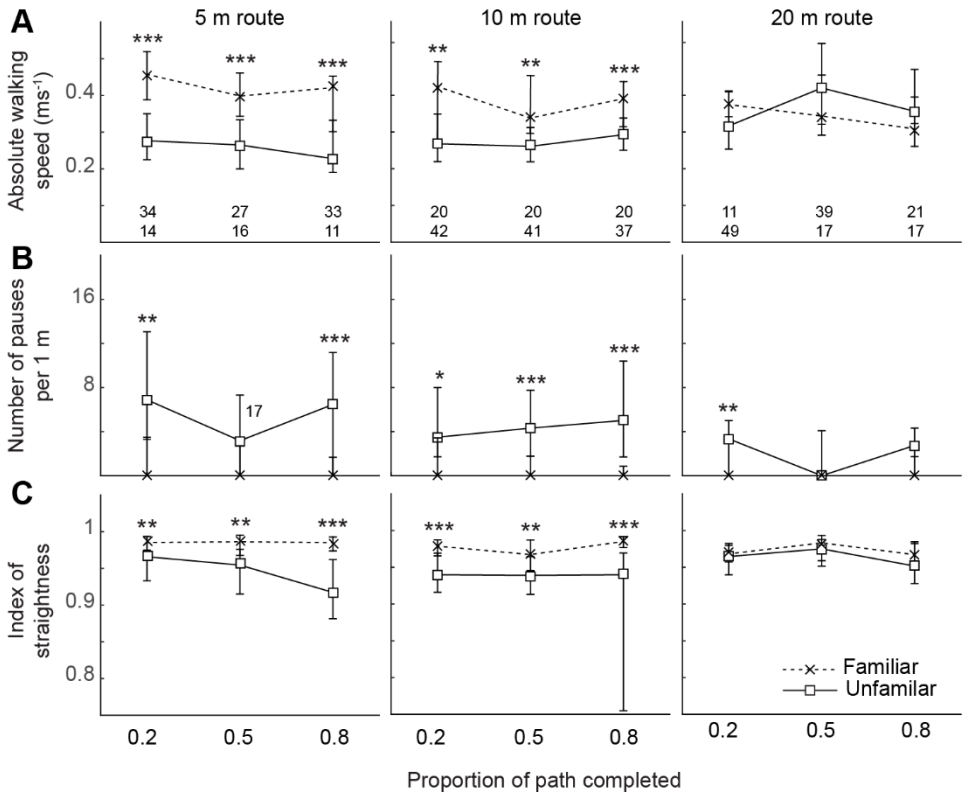

Figure S1:

Path disturbance caused by changes in the visual surroundings. Ants with either a 5 m (left), 10 m (middle) or 20 m (right) homeward route were released such that they encountered novel visual cues when having completed either 20%, 50% or 80% of their homing path (see Fig. 1B for experimental setup). Paths were recorded with a high-speed camera. **A)** Test ants that were unfamiliar with the visual cues walk slower than control ants that were familiar with the visual cues. Speed values are plotted as medians with error bars showing 25th and 75th percentiles. Asterisks show significant differences between test and control ants using Mann-Whitney tests (*, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$). Numbers next to error bars show number of ants per group (sample sizes are only shown in B and C if different). **B)** As (A) but for number of pauses per 1 m path. **C)** As (A) but for index of straightness.

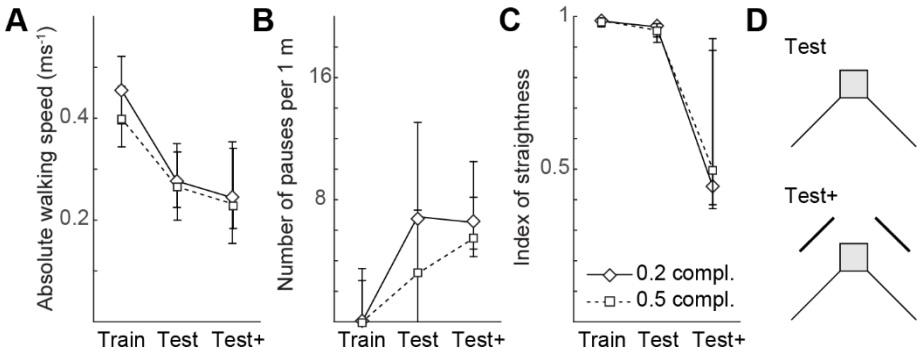

Figure S2:

Path characteristics for tests with different levels of visual novelty. Ants with either 20 % or 50 % of their 5 m paths completed were released in a control condition (Train), with novel visual setup (Test) or with a novel visual set-up with increased visual change (Test+). Paths were recorded with a high-speed camera. **A)** Speed values are plotted as medians with error bars showing 25th and 75th percentiles. 0.2 of path completed: n = 34 ants, 14 ants, 13 ants (Train, Test, Test+). Kruskal-Wallis test with Dunn's multiple comparison tests, Train vs Test, $P < 0.001$, Train vs Test+, $P < 0.001$, Test vs Test+, $P \geq 0.05$. 0.5 of path completed: n = 27 ants, 16 ants, 10 ants. Train vs Test, $P < 0.001$, Train vs Test+, $P < 0.01$, Test vs Test+, $P \geq 0.05$. **B)** As (A) but for number of pauses per 1 m path. 0.2 of path completed: n = 34 ants, 14 ants, 14 ants. Kruskal-Wallis test with Dunn's multiple comparison tests, Train vs Test, $P < 0.01$, Train vs Test+, $P < 0.001$, Test vs Test+, $P \geq 0.05$. 0.5 of path completed: n = 27 ants, 17 ants, 10 ants. Train vs Test, $P \geq 0.05$, Train vs Test+, $P < 0.001$, Test vs Test+, $P \geq 0.05$. **C)** As (A) but for index of straightness. 0.2 of path completed: n = 34 ants, 14 ants, 13 ants. Kruskal-Wallis test with Dunn's multiple comparison tests, Train vs Test, $P < 0.05$, Train vs Test+, $P < 0.001$, Test vs Test+, $P \geq 0.05$. 0.5 of path completed: n = 27 ants, 16 ants, 10 ants. Train vs Test, $P < 0.05$, Train vs Test+, $P < 0.001$, Test vs Test+, $P \geq 0.05$. **D)** Experimental configuration for test with novel visual setup (Test) or with a novel visual set-up with increased visual change (Test+). For details see Fig. 1B.

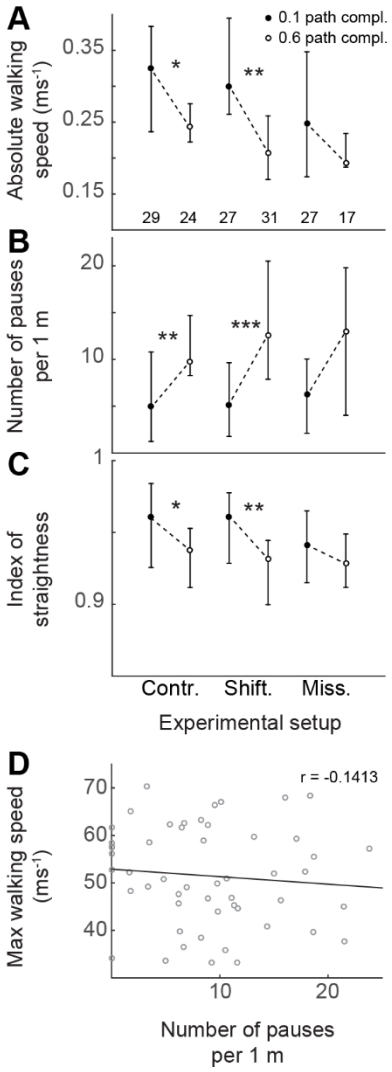

Figure S3:

Ants respond more to familiar visual cues when further along their homing path. Homing ants had completed 10% or 60% of the homing path when reaching the channel exit (see Fig. 1C for experimental setup). Paths were recorded with a high-speed camera. **A)** Speed values are plotted as medians with error bars showing 25th and 75th percentiles. Asterisks show significant differences between groups using Mann-Whitney tests (*, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$). Numbers below error bars show number of ants per group. **B)** As (A) but for number of pauses per 1 m path. **C)** As (A) but for index of straightness. **D)** Max speed values from all control ants are plotted against the frequency of pauses. Spearman rank correlation, $P \geq 0.05$.